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Morphological analysis of the cloacal region in *Xenopus laevis* embryos

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Initiation of development and differentiation of the alimentary canal involves many complex processes. The development of the cloacal region in particular, is not well understood and abnormalities in this region result in a variety of anorectal malformations, which are disabling for the newborn. The amphibian alimentary canal, a useful developmental model, develops in three parts: stomodeum, mesenteron and proctodeum. The stomodeum and proctodeum develop at the extreme anterior and posterior regions of the embryo respectively, and are the only two areas in the developing embryo where the ectodermal and endodermal germ layers are directly juxtaposed without intervening mesoderm. Molecular studies have been conducted involving the proctodeal region. However, morphological analysis of this region appears to be limited in the current literature. This study aims to investigate and elucidate morphological features present during the development of the proctodeal region and dissolution of the cloacal membrane in *Xenopus laevis* embryos. *Xenopus laevis* frogs were mated and the resulting embryos were collected, allowed to develop further in a suitable environment and staged according to Nieuwkoop and Faber (1967). For all the techniques used, a minimum of six specimens at each stage were analysed. Staged specimens were fixed in 10% formalin, routinely processed and embedded in JB-4 resin for histological evaluation. Serial sections were cut at 6μm and stained with Gill's haematoxylin and eosin technique, to examine the general structure and pin-point dissolution of the cloacal membrane. Confocal microscopy was used for optical fluorescent sectioning to view interior structures. Specimens for confocal microscopy were fixed in Bouin's fixative. For scanning electron microscopy, embryos were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and routinely processed. Three dimensional images of the proctodeal region were obtained using the scanning electron microscope. Initially at the caudal end of the embryo, the ectoderm invaginates (stage 21) forming a small depression, known as the proctodeum. There is apposition of the outer ectoderm and inner endoderm. Low cilia are found dispersed evenly across the surface of the embryo. As development continues, from stage 21 to stage 23, the ectodermal depression deepens and the ectoderm and endoderm become reduced in thickness to form the cloacal membrane. The cells around the proctodeum project out forming a slight bulge. The cilia become more numerous and appear more concentrated around the anal region. The depression increases and at stage 24 there is perforation of the membrane, producing a free passage of communication from the hindgut to the exterior. From stage 24 to stage 26 the perforation increases in width and becomes more apparent. Molecular analysis of this region is ongoing. Morphological evidence combined with molecular analysis will provide a greater understanding of the development of the proctodeal region and dissolution of the cloacal membrane.

Nieuwkoop PD, Faber J (1967) Normal Table of *Xenopus laevis* (Daudin): A systematical and Chronological survey of the Development from the fertilized egg till the end of metamorphosis. Eds. North-Holland Publishing Company, Amsterdam.

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